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19 ABSTRACT (Continue on reverse if necessary and identify by block number) The goal of this project is to assemble a systematic framework for design and construction of synthetic metal-binding proteins for metal recognition and recovery. In the first phase of this work, we are investigating metal-binding sites that can be built into the surface of a protein with relatively few amino acid substitutions. One such site, consisting of two histidines positioned His-X-X-X-His in an alpha-helix, has been built into yeast iso-1-cytochrome c. The resulting mutant protein binds Cu(II) and other metals with high affinity. In addition to the protein engineering work, a novel technique for quantifying metal-protein interactions has been developed. This technique is based on the partitioning of metal-binding proteins between two aqueous-polymer phases. When polymer in one phase is derivatized with the appropriate metal chelate (e.g. poly (ethylene glycol)-IDA-Cu(II)), partitioning of a protein between the two phases depends on the number of binding sites and the affinity of each site for the metal. We have (cont.)			
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demonstrated that metal-protein binding constants can be obtained for binding sites accessible to the chelated metals.

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DATE: JUNE 21, 1989

PROGRESS REPORT ON CONTRACT N00014-88-K-0469

PRINCIPAL INVESTIGATOR: Frances H. Arnold

CONTRACTOR: California Institute of Technology

CONTRACT TITLE: Design and Construction of Synthetic Metal-Binding Proteins

START DATE: July 1, 1988

RESEARCH OBJECTIVE: Investigate protein-metal interactions and construct metal-binding sites on proteins by site-directed mutagenesis.

PROGRESS (Year 1): A novel method for studying and quantifying interactions between proteins and metal chelates has been developed. The method entails the use of a two-phase aqueous system in which one polymer (e.g. poly(ethylene glycol) is derivatized with a metal chelate. We have shown that protein-metal binding constants can be obtained from partitioning experiments using these metallated polymers [see publications].

Synthetic metal-binding variants of bovine growth hormone yeast iso-1-cytochrome c has been constructed by site-directed mutagenesis at Caltech (cytochromec) and at Monsanto Co. (BGH). By introducing two histidines as positions 4 and 8 in the N-terminal alpha-helix, a cytochrome with a high affinity for Cu(II) has been made and purified. Partitioning experiments on the bovine growth hormone indicate that this His-X-X-X-His site in the helix has an affinity for Cu(II) in IDA (iminodiacetic acid) that is roughly ten times higher than the binding constant for a single histidine with Cu(II)-IDA. In other words, the addition of two surface-accessible histidines in this chelating arrangement is equivalent to the addition of ten histidines that do not chelate.

WORK PLAN (Year 2): In year 2 we will continue to construct and characterize metal-binding cytochromes c, in a effort to determine the parameters important in building synthetic metal binding sites into proteins. The ability of the His-X-X-X-His and other mutants to bind metals other than Cu(II) will be evaluated. We hope to develop a quantitative understanding of the relationship between the placement of specific metal ligands (individual amino acids) and the affinities exhibited for various metal ions.

PUBLICATIONS AND REPORTS (Year 1):

1. A paper describing the new method for measuring protein-metal interactions has been submitted for publication. The protein engineering work is very recent--a manuscript should be available within a few months.

Suh, S-S. and F. H. Arnold (1989) "A Mathematical Model for Metal Affinity Protein Partitioning," submitted to Biotechnology and Bioengineering. (copy enclosed)

2. Frances Arnold has been invited to present these results at the following meetings:

6th International Conference on Aqueous Two-Phase Partitioning, August, 1989.
Abstract title: Metal Affinity Aqueous Two-Phase Extraction.

1989 Gordon Conference on Reactive Polymers, Ion Exchangers, and Adsorbents,
July, 1989. Abstract title: Protein Engineering and Metal Affinity Separations.

UCLA Symposium on Protein Purification and Biochemical Engineering, March,
1990.

3. The Annual Report will be distributed to the ONR Distribution List as required within
the next month.

TRAINING ACTIVITIES: Two graduate research assistants have been working on the
site-directed mutagenesis and protein characterization. One is an NSF Predoctoral Fellow
(Chemistry), while the other is a chemical engineering graduate student.

Women or minorities	-2
Non-citizens	-0

AWARDS/FELLOWSHIPS: Frances Arnold received a Presidential Young Investigator
Award from NSF, April, 1989.

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